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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TARTRAZINE AND SUNSET YELLOW IN FOODSTUFF

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ABSTRACT

A simple, rapid, precise and highly selective spectrophotometric method was developed for simultaneous estimation of Tartrazine and Sunset yellow in pure as well as foodstuffs. The simultaneous equation method is based on measurement of absorbance at 427nm and 483 nm as two wavelengths selected for quantification of Tartrazine and Sunset yellow using distilled water as a solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. A double-beam shimadzu UV-visible spectrophotometer, 1800 with a pair of 1 cm matched quartz cells was used to measure the absorbance of the solutions in developed method. The method was validated as per ICH guidelines. Linearity ranges from 5-25µg/ml for Tartrazine and 5-25 µg/ml for Sunset yellow of the dyes. % RSD calculated was less than equal to 2 which indicates accuracy and reproducibility of the method. Recovery study indicates that these drugs could be quantified simultaneously without interference of excipient present in formulation. The developed UV spectroscopic method is suitable for the analysis of TAR and SY in combined foodstuffs.

KEYWORDS

Tartrazine, Sunset yellow, Simultaneous Equation, Method Validation and UV Spectrophotometer.

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INTRODUCTION

Food additives are commonly used in processed foodstuffs to improve appearance, flavor, taste, colour, texture, nutritive value and conservation. Since the visual aspects is an important factor for the selection of products by consumers. When compared to natural dyes, synthetic dyes show several advantages such as high stability to light,

oxygen and pH, colour uniformity, microbiological contamination, relatively lower production costs, etc[1]. Tartrazine (TAR, E-102) {trisodium; 5-oxo-1-(4-sulfonatophenyl)-4-[(sulfonatopheny) diazenyl]-4H-pyrazole-3-carboxylate} and Sunset yellow (SY, E-110) {disodium; 6-oxo-5-[(4-sulfonatophenyl) hydrazinylidene] naphthalene-2-sulfonate} are synthetic dyes which are added to many food products[2,3].

The presence and content of these dyes must be controlled due to their potential harmfulness to human beings. Due to its toxicity, especially when consumed in excess, synthetic dyes are strictly controlled by laws, regulations and acceptable daily intake (ADI) values for food safety[4,5] and ADI for Tartrazine and Sunset Yellow is 7.5mg/kg and 2.5mg/kg of body weight respectively[6].

The Food Safety and Standards Authority of India (fssai) has been established under Food Safety and Standards, 2006 created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption. It has issued comprehensive schemes that regulating the use of food colours and their allowed levels in all food products[7].

The maximum level of Tartrazine and Sunset yellow dyes should not be more than 100ppm of the final food for consumption[8]. Thus, it is necessary to develop accurate and reliable analytical methods for the confirmative determination of synthetic food dyes i.e. TAR and SY in foodstuffs to ensure food safety and consumer health.

A large number of analytical methods for food colours have been proposed but the present work deals with to develop simple and accurate spectrophotometric method for simultaneous determination of Tartrazine and Sunset yellow from foodstuff.

MATERIAL

Pure standards of TAR and SY were obtained as gift sample and their marketed foodstuff was purchased from the market. Distilled water of analytical grade was used as the solvent. A double-beam shimadzu UV- visible spectrophotometer, 1800 with a pair of 1 cm matched quartz cells were used to measure the absorbance of the solutions.

Sample

Custard powder (Pillsbury)

UV Spectroscopic Method

Preparation of Standard Stock Solution

The standard stock solution of Tartrazine (TAR) and Sunset yellow (SY) was prepared by transferring accurately weighed 10 mg of Tartrazine and Sunset yellow Separately into 10 ml volumetric flask containing distilled water. Then volume was made up to the mark by using distilled water to give a concentration of 1000µg/ ml. From this, 1ml of the solution was transferred to a 10ml volumetric flask and make up the volume with distilled water to give a concentration of each 100 µg/ml, which is a standard stock solution and it is further diluted with distilled water to get concentration range of 10µg/ml of each Tartrazine(TAR) and Sunset yellow(SY).

Determination of absorption maxima

The prepared standard solutions (10µg/ml) were scanned in the UV-VIS spectrophotometer in the wavelength range of 400-800 nm and an overlain spectrum was recorded. Using the overlain spectra, the wavelength maxima of both dyes, i.e. 427 nm (λ_1 for TAR) and 483 nm (λ_2 for SY), were selected as two sampling wavelengths for simultaneous equation method. The prepared stock solutions were then diluted to get the solution of 5-25 µg/ml and 5-25µg/ml for Tartrazine and Sunset yellow respectively. The absorbance of these solutions were measured at the selected wavelengths and absorptivities were determined (Table No.1).

Vierodt's Method of Simultaneous Equations

This method is based on absorption of drugs at the wavelength maximum of the other. The concentrations of the drugs were calculated from the following equations:

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2} \quad \text{.....Eq. 1}$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2} \quad \text{.....Eq. 2}$$

Where, A_1 and $A_2$are absorbance of mixture at 427 nm and 483 nm respectively, ax_1 and ax_2 are

absorptivities of TAR at λ_1 and λ_2 respectively, ay_1 and ay_2 are absorptivities of SY at λ_1 and λ_2 respectively. C_x and C_y are the concentrations of TAR and SY respectively.

Sample preparation

5 gm of custard powder was weighed and transferred to 50 ml volumetric flask containing distilled water. Then above solution was filtered through0.45µ whatmann filter paper. After filtration, from this 5 ml was taken and diluted upto 10 ml with distilled water.

Absorbance of sample solutions was recorded at 427nm and 483 nm and then concentration of both the dyes were calculated using Equation 1 and 2 and the results are given in Table No.2.

METHOD VALIDATION

The developed method was validated as per ICH guidelines for the following parameters:

Linearity

From the each 'Std Stock TAR' (1000µg/ ml) 1 ml and 'Std Stock SY' (1000µg/ml) 1 ml and made up to the volume 10 ml with distilled water to make the conc. of TAR100µg/ml and 100µg/ml.

From this solution 0.5, 1, 1.5, 2, 2.5 ml for TAR and 0.5, 1, 1.5, 2, 2.5 ml for SY were transferred in a series of 10 ml volumetric flasks. The volume was made up to the mark with distilled water to obtain the concentration of 5, 10, 15, 20, 25µg/ml and 5, 10, 15, 20, 25µg/ml for TAR and SY respectively.

Calibration curves of TAR and SY was constructed by plotting the Absorbance of TAR v/s Conc. of TAR and Absorbance of SY v/s Conc. of SY. The correlation coefficient (r^2) of least square linear regression for TAR and SY was calculated.

Range

The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

Accuracy

Recovery study was carried out by the standard addition method by adding a known amount of TAR and SY to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 1 ml of sample solution was transferred to 4 different 10 ml volumetric flasks (labelled as blank, 80%, 100%, 120%) separately and 0, 8, 10, 12µg/ml standard solution was added respectively and the volume was made up to the mark with distilled water. Absorbances were noted for these samples. Standard deviation and % RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below:

% Recovery = Observed value / True value ×100

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision)

Intraday precision was determined by analyzing the drugs at concentration (10µg/ml) for both the drugs and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (10µg/ml) for both the drugs. Absorbance of each was measured.

Robustness

The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of Detection

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of TAR

(10µg/ml) and SY (10µg/ml) and LOD calculated by LOD = 3.3 (SD/S) Where, SD- standard deviation; S= slope of the curve.

Limit of Quantification

Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution SX (6µg/ml) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

RESULTS AND DISCUSSION

Linearity

The linearity of this method was determined at ranges from 5-25µg/ml and 5-25µg/ml for TAR and SY respectively. The regression equation was found to be.

Accuracy

The accuracy of the analytical method for TAR and SY was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 427 nm and 483 nm results were expressed in terms of % recoveries.

Precision

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that method was highly precise.

Preliminary Analysis of Tartrazine and Sunset yellow

Preliminary analysis of Tartrazine and Sunset yellow such as description, solubility was performed and it was found that, the Tartrazine and Sunset yellow was soluble in water, methanol.

Assay of sample of foodstuff

Amount of dyes present in marketed foodstuff was calculated using simultaneous equation at 427 nm and 483 nm for TAR and SY Respectively, and $y=0.0496x - 0.0148$ and $y= 0.039x + 0.008$ for TAR and SY respectively. The tartrazine and sunset yellow in the tested foodstuff samples are in compliance with FSSAI limit (200 PPM).

Summary and conclusion

Summary of UV Spectrophotometric Method for Tartrazine and Sunset yellow.

Table No.1: Absorptivity of TAR and SY at 427 nm, 483 nm respectively

S.No	Components (10µg/ml)	Absorptivity at 427 nm	Absorptivity at 483 nm
1	TAR	0.482	0.139
2	SY	0.377	0.172

Table No.2: Result analysis of the foodstuff

S.No	Dyes	Label Claim	Amount found
1	TAR	NMT 200 ppm	177.7 ppm
2	SY	NMT 200 ppm	86.9 ppm

Table No.3: Linearity of Tartrazine and Propranolol Hydrochloride

	Tartrazine (TAR)		Sunset yellow (SY)	
S.No	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	5	0.232	5	0.204
2	10	0.479	10	0.388
3	15	0.731	15	0.605
4	20	0.981	20	0.794
5	25	1.22	50	0.977
6	Regression equation: $y= 0.0496x - 0.0148$		Rregression equation:$y= 0.039x + 0.008$	
7	R^2= 0.999		R^2=0.999	

Table No.4: Table for accuracy

S.No	Drug	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Amount Recovered (µg/ml)	%Recovery
1	TAR	10	80% (8µg/ml)	11.26	98.75
		10	100% (10µg/ml)	13.14	99.54
		10	120% (12µg/ml)\7	15	101.13
2	SY	10	80% (8µg/ml)	9.9	96.66
		10	100% (10µg/ml)	12.09	99.58
		10	120% (12µg/ml)	14.12	99.64

Intra-day Precision

Table No.5: Intra-day precision

S.No	TAR		SY	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.482	10	0.377
2	10	0.481	10	0.375
3	10	0.482	10	0.372
4	10	0.483	10	0.377
5	10	0.481	10	0.376
6	% RSD	0.173	%RSD	0.552

Inter-day Precision

Table No.6: Inter-day precision

S.No	TAR		SY	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.482	10	0.377
2	10	0.483	10	0.376
3	10	0.481	10	0.372
4	10	0.482	10	0.377
5	10	0.481	10	0.375
6	% RSD	0.173	%RSD	0.552

Repeatability

Table No.7: Repeatability study

S.No	TAR		SY	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	10	0.482	10	0.377
2	10	0.483	10	0.375
3	10	0.481	10	0.376
4	10	0.483	10	0.377
5	10	0.483	10	0.375
	% RSD	0.185	%RSD	0.265

Limit of Detection

Table No.8: For Limit of Detection

S.No	LOD (µg/ml)	Conc.
1	TAR	0.059 µg/ml
2	SY	0.084µg/ml

Limit of Quantification

Table No.9: For Limit of Quantification

S.No	LOQ (µg/ml)	Conc.
1	TAR	0.180 µg/ml
2	SY	0.256 µg/ml

Ruggedness

Table No.10: Ruggedness

S.No	Wavelength	Absorbance	
		TAR(10 µg/ml)	SY(10 µg/ml)
1	Wavelength 1	0.479	0.374
2	Wavelength 2	0.477	0.372
3	Wavelength 3	0.472	0.373
4	%RSD	0.75	0.268

Robustness

Table No.11: Robustness

S.No	Analyst	Absorbance	
		TAR(10 µg/ml)	SY(10 µg/ml)
1	Analyst 1	0.482	0.377
2	Analyst2	0.482	0.376
3	Analyst3	0.483	0.377
4	%RSD	0.119	0.153

Table No.12: For Summary

S.No	Parameters	Values	
		TAR	SY
1	Beer's Law limit (µg/ml)	5-25	5-25
2	Absorption maxima (nm)	427	483
3	Standard regression equation	*y=0.0496x - 0.0148*	*y= 0.039x + 0.008*
4	Correlation coefficient (R^2)	0.999	0.999
5	Accuracy	98-101%	96-99%
6	Precision (% RSD) Repeatability	0.185	0.265
7	LOD (µg/ml)	0.059 µg/ml	0.084 µg/ml
8	LOQ (µg/ml)	0.180 µg/ml	0.256 µg/ml
9	Robustness (%RSD)	0.119	0.153
10	Ruggedness	0.75	0.268

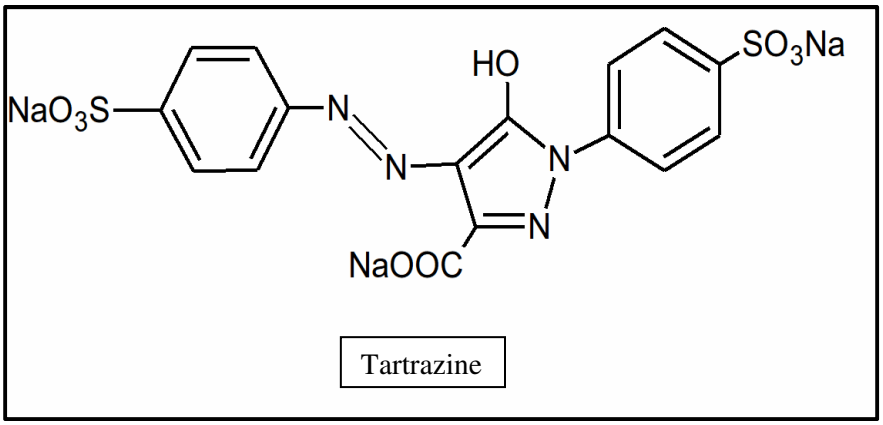

Figure No.1: Structure of TAR

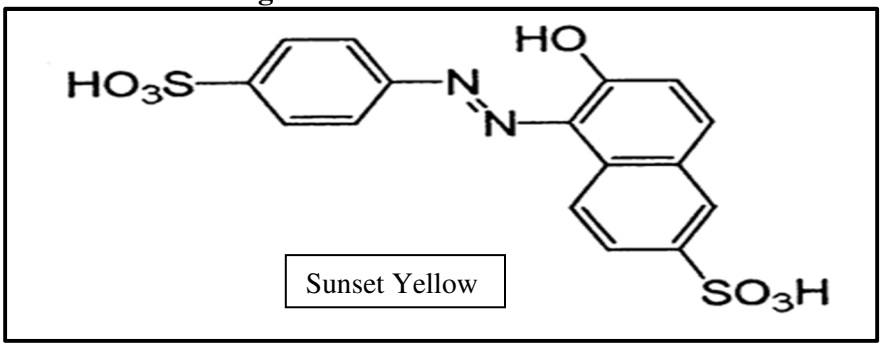

Figure No.2: Structure of SY

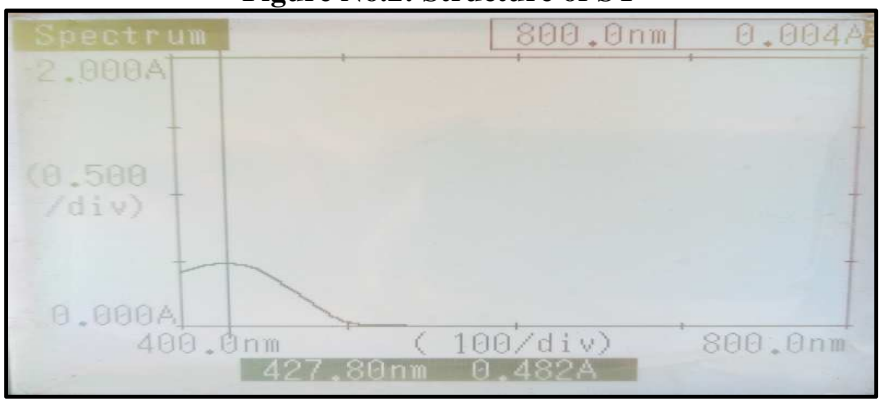

Figure No.3: spectra showing absorption maxima of TAR at 427 nm

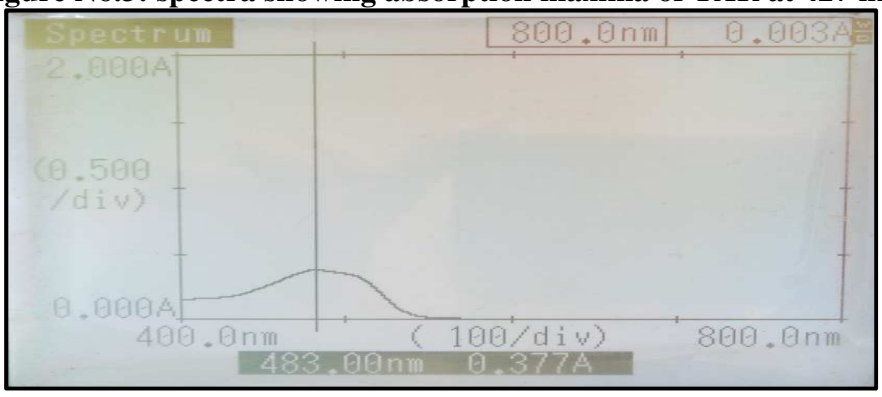

Figure No.4: spectra showing absorption maxima of SY at 483 nm

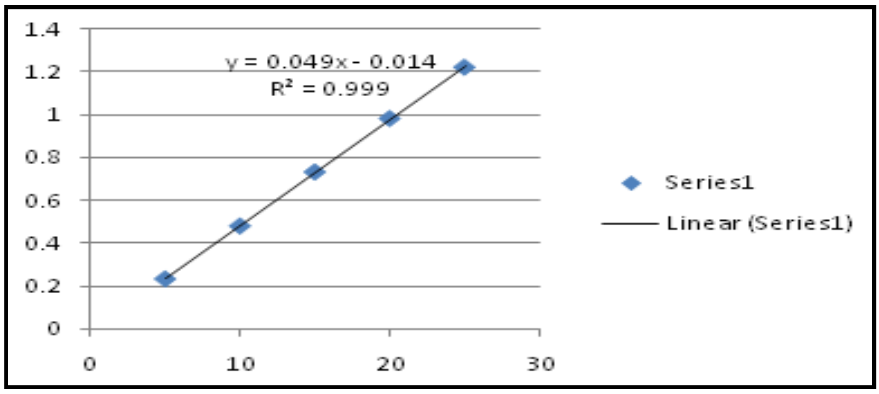

Figure No.5: Linearity of TAR

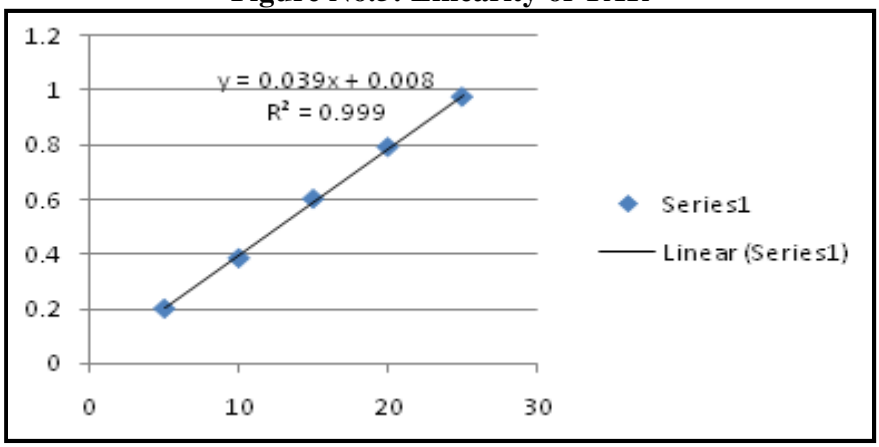

Figure No.6: Linearity of SY

CONCLUSION

The UV-Spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Tartrazine and Sunset yellow in pure form and its marketed fodstuffs form without any interference from the excipients. This method can be effectively applied for the routine analysis of Tartrazine and Sunset yellow in foodstuffs. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

ABBREVIATIONS

UV-Ultra Violet
TAR- Tartrazine
SY- Sunset yellow
LOD- Limit of Detection
LOQ- Limit of Quantification

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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